APPENDIX

Changes to Specification:

The following is a marked-up version of the amended paragraphs:

Page 21, lines 17-31:

The introduction of nonsilent mutations, with the aid of PCR techniques, into one or more cassettes of the modular gene previously defined, led to genes encoding polymerases exhibiting an amino acid sequence differing by at least one amino acid with respect to the T7 expressed from the modular gene. Mutant genes were in particular prepared which encode at least one modified amino acid in the B motif of the wild-type enzyme, for example with an alanine (A) in place of an arginine (R) at position 627 and/or an alanine (A) in place of a serine (S) at position 628 and/or an alanine (A) in place of a lysine ~~(A)~~ (K) at position 631 and/or an alanine (A) in place of an arginine (R) at position 632 and/or an alanine (A) in place of a tyrosine (Y) at position 639.

Page 30, line 5 to page 31, line 24:

The reactions are performed in 20 µl of a buffer derived from that described by J.F. Milligan, D.R. Groebe, G.W. Witherell, O.C. Uhlenbeck, Nucleic Acids Res. ~~2~~15, 8783 (1987), namely Tris-HC1 40 mM, pH 8.1, spermidine 1 mM, PEG 8% (g/V), TRITON X-100 (~~a surfactant~~Octoxynol-9) 0.01% (V/V), BSA 5 µg/100 µl, 1 µl (40 u) of porcine RNAguard (Pharmacia Biotech), UTP 12.5 µM, a 32P UTP 0.5 µCi (Amersham, 10 mCi/ml 400 Ci/mmol) 0.4 mM of the three ribonucleoside triphosphates A, G, C, $Mg(OAc)_2$ 6 mM. The template concentration is set at 10^{11} copies of each strand in 20 µl of reaction. The wild-type T7 RNA polymerase is used at 0.5 µM (100 ng/20 µl), the mutated T7 RNA polymerase R627A at 3.65 µM (730 ng/20 µl). Before adding the enzymes, the reactions are denatured for 5 minutes at 65°C in a heating block and then gradually brought to 37°C. The reactions are initiated by the addition of the polymerases, incubated for 1 hour at 37°C and then

stopped by the addition of an equal volume of 2× blue formamide (formamide 90%, EDTA 25 mM, xylene cyanol 0.02%, bromophenol blue 0.02%) and denatured for 5 minutes at 95°C. 20 µl of each reaction are deposited on a denaturing gel (20% acrylamide, urea 7 M, 1X TBE), and then after migration, the gel is autoradiographed at -70°C on a Biomax MR film (Kodak). The results (electrophoretic profiles) are presented in Figure 5, and in particular the transcription results obtained with the mutated T7 RNA polymerase R627A (wells 1-3) and the wild-type T7 RNA polymerase (wells 4-6), on the single-stranded RNA templates (wells 1 and 4), double-stranded DNA (wells 2 and 5), and single-stranded DNA (wells 3 and 6). The transcription on single-stranded RNA, detected by detection of a complete transcript of 33 bases, is possible using the mutated T7 RNA polymerase R627A (well 1) and not the wild-type enzyme (well 4) which produces on the other hand many abortive transcripts; see nevertheless the different results obtained in Example 3 below. The mutated T7 RNA polymerase R627A exhibits a residual transcription activity on double-stranded DNA (well 2), characterized by the presence of a predominant transcript which is smaller in size than the expected transcript, and the presence of a small quantity of abortive products. On single-stranded DNA (well 3), this transcript of abnormal size disappears, whereas the quantity of abortive products increases. By contrast, the wild-type enzyme allows the production of specific transcripts in the presence of DNA templates (wells 5 and 6), this enzyme exhibiting, moreover, a better transcription activity on the double-stranded DNA template (well 5) than on the single-stranded DNA template (well 6); for these two templates, the wild-type enzyme induces the synthesis of numerous abortive transcripts. These results show that the replacement of the arginine 627 by an alanine confers on the mutant enzyme the possibility of synthesizing RNA from an RNA template and induces the loss of capacity to synthesize RNA from a DNA template.

Changes to Claims:

Claim 42 is canceled.

The following is a marked-up version of the amended claims:

35. (Amended) A method of amplifying an RNA target sequence, by transcription under the control of a promoter, in an RNA sample comprising said target sequence, said method comprising bringing said sample into contact:

- with a reagent capable of hybridizing with RNA comprising said target sequence,
- in the absence of deoxyribonucleoside triphosphates,
- and with an enzymatic system comprising an RNA-dependent RNA polymerase ~~activity~~, under conditions allowing the hybridization of said reagent with said RNA comprising said target sequence and under conditions allowing the functioning of said RNA-dependent RNA polymerase ~~activity~~;

wherein said reagent contains:

(i) a first nucleotide strand comprising: a) a first nucleotide segment capable of playing the role of sense strand of a promoter for said RNA polymerase ~~activity~~ and b) downstream of said first segment, a second nucleotide segment comprising a sequence capable of hybridizing with a region of said RNA, and

(ii) in the hybridized state on the first strand, a second nucleotide strand comprising a third nucleotide segment capable of hybridizing with said first segment so as to form with it a functional double-stranded promoter;

~~and~~ wherein said RNA polymerase ~~activity~~ (1) is from a family of RNA polymerases whose promoters have a consensus sequence from position -17 to position -1 and (2) is capable of transcribing an RNA template, in the presence of said reagent hybridized with said template, in the absence of associated protein factor and in the absence of a ligase activity.

43. (Amended) A method according to claim ~~42~~35, wherein said RNA polymerase is from a family of RNA polymerases selected from the group consisting of T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase.

44. (Amended) A method according to claim ~~42~~35, wherein said RNA polymerase is derived by mutation from an RNA polymerase from a family of RNA polymerases selected from the group consisting of T7, T3 and SP6 RNA polymerases.

48. (Amended) An RNA polymerase, capable of transcribing, under the control of a promoter, a polynucleotide target of interest of a sequence contained in a polynucleotide template, by synthesizing, in the presence of said template and in the absence of associated protein factor and in the absence of a ligase activity, a product of transcription containing an RNA sequence complementary to said sequence, ~~and~~ wherein said RNA polymerase ~~being~~ (1) is capable of synthesizing said product of transcription with a better yield when said target sequence of said template consists of RNA than when it consists of DNA and (2) is from a family of RNA polymerases whose promoters have a consensus sequence from position -17 to position -1.

59. (Amended) An RNA polymerase according to claim 58, ~~wherein said amino acid replaced is an arginine or a lysine and/or~~ wherein said other amino acid residue is an alanine, valine, leucine, isoleucine, glycine, threonine or serine residue.